

UNCLASSIFIED
SECTION 7 CLASSIFICATION OF THIS PAGE

MASTER COPY

FOR REPRODUCTION PURPOSES-

(2)

REPORT DOCUMENTATION PAGE FILE COPY

AD-A214 088

1b. RESTRICTIVE MARKINGS					
			3. DISTRIBUTION/AVAILABILITY OF REPORT Approved for public release; distribution unlimited.		
4. PERFORMING ORGANIZATION REPORT NUMBER(S)			5. MONITORING ORGANIZATION REPORT NUMBER(S) ARO 24428.5-LS		
6a. NAME OF PERFORMING ORGANIZATION Duke University Medical Center		6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION U. S. Army Research Office		
6c. ADDRESS (City, State, and ZIP Code) Duke University Medical Center Durham, NC 27706			7b. ADDRESS (City, State, and ZIP Code) P. O. Box 12211 Research Triangle Park, NC 27709-2211		
8a. NAME OF FUNDING/SPONSORING ORGANIZATION U. S. Army Research Office		8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER DAAL03-86-K-0130		
8c. ADDRESS (City, State, and ZIP Code) P. O. Box 12211 Research Triangle Park, NC 27709-2211			10. SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO. PROJECT NO. TASK NO. WORK UNIT ACCESSION NO.		
11. TITLE (Include Security Classification) Excitation by Odorants of Olfactory Receptor Cells: Molecular Interaction at the Ciliary Membrane					
12. PERSONAL AUTHOR(S) Robert H. Anholt					
13a. TYPE OF REPORT Reprint		13b. TIME COVERED FROM TO		14. DATE OF REPORT (Year, Month, Day)	
15. PAGE COUNT					
16. SUPPLEMENTARY NOTATION The view, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy, or decision, unless so designated by other documentation.					
17. COSATI CODES FIELD GROUP SUB-GROUP			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)		
19. ABSTRACT (Continue on reverse if necessary and identify by block number)					
20. DISTRIBUTION/AVAILABILITY OF ABSTRACT <input type="checkbox"/> UNCLASSIFIED/UNLIMITED <input type="checkbox"/> SAME AS RPT. <input type="checkbox"/> DTIC USERS					
21. ABSTRACT SECURITY CLASSIFICATION Unclassified					
22a. NAME OF RESPONSIBLE INDIVIDUAL			22b. TELEPHONE (Include Area Code)		22c. OFFICE SYMBOL

DD FORM 1473, 84 MAR

83 APR edition may be used until exhausted.
All other editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE
UNCLASSIFIED

89 10 30 147

AR0 24428.5-LS

CHEMICAL SENSES

Volume 1 Receptor Events and Transduction in Taste and Olfaction

edited by

Joseph G. Brand

*Monell Chemical Senses Center
Veterans Administration Medical Center
and University of Pennsylvania
Philadelphia, Pennsylvania*

John H. Teeter

*Monell Chemical Senses Center
and University of Pennsylvania
Philadelphia, Pennsylvania*

Robert H. Cagan

*Colgate-Palmolive Company
Piscataway, New Jersey*

Morley R. Kare

*Monell Chemical Senses Center
and University of Pennsylvania
Philadelphia, Pennsylvania*

Marcel Dekker, Inc.

New York and Basel

Copyright © 1989 by Marcel Dekker, Inc.

Excitation by Odorants of Olfactory Receptor Cells

Molecular Interactions at the Ciliary Membrane

Robert R. H. Anholt, R. William Farmer, and
Christa A. Karavanich
Duke University Medical Center
Durham, North Carolina

I. INTRODUCTION

The first physiological measurements of the response of the olfactory epithelium to odorants were published in 1956 by Ottoson, who introduced the "electroolfactogram" (EOG), a negative-voltage transient that can be recorded from the epithelium after odor application (Fig. 1a; Ottoson, 1956). The EOG is believed to result from the summated activity of individual olfactory neurons. Prolonged application of odorants results in the appearance of a transient response, the "phasic" response, followed by a steady-state potential, the "tonic" response (Fig. 1b). Initial characterization of the EOG showed that it could be obtained only from olfactory and not from respiratory epithelium (Ottoson, 1956), that the amplitudes of both the phasic and the tonic responses were dependent on the concentration of odorant (Ottoson, 1956), and that the EOG was abolished after zinc sulfate lesions of the olfactory epithelium or removal of olfactory cilia by treatment with Triton X-100 (Adamek et al., 1984; Bronshtein and Minor, 1977). Further studies on the EOG indicated that sodium and potassium are the main ions carrying the currents (Tagaki et al., 1968; 1969) and that calcium is essential for generation of the EOG (Suzuki, 1978). Later studies showed that cyclic nucleotides and phosphodiesterase inhibitors could modulate the EOG (Menevse

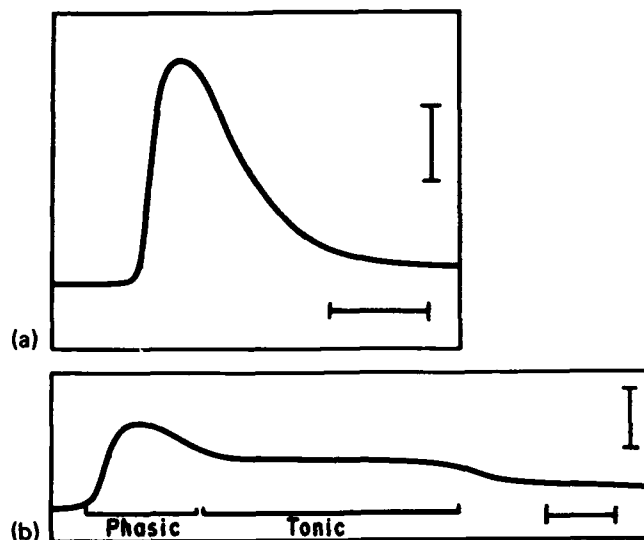


FIGURE 1 Electrical responses of the olfactory epithelium to odorants. (a) Typical shape of a monophasic electroolfactogram (EOG) after application of 0.5 mL of odorized air to the olfactory epithelium of the frog. The stimulus is butanol. Vertical bar: 1 mV; time bar: 2 seconds. (b) Biphasic shape of the EOG showing a phasic and a tonic component. The EOG was recorded during continuous stimulation of the olfactory epithelium with odorized air equilibrated with 0.01 M butanol for 15 seconds. Vertical bar: 1 mV; time bar: 3 seconds. (Modified from Ottoson, 1956, with permission.)

et al., 1977; Minor and Sakina, 1973) and that adenylate cyclase could be enzymatically identified in olfactory tissue (Kurihara and Koyama, 1972). Thus, early electrophysiological measurements indicated the involvement of ion channels, adenylate cyclase, and calcium in the generation of the macroscopic response of the olfactory epithelium to odorants.

In this chapter, we examine these parameters at the molecular level and describe how odorant-sensitive ion channels, cAMP, and calcium may act in concert to mediate and regulate excitation of olfactory receptor neurons by odorants.

II. DETACHMENT OF CHEMOSENSORY CILIA FROM OLFACTORY EPITHELIUM

A substantial body of evidence has accumulated indicating that initial transduction events in olfaction occur at the chemosensory cilia (Anholt, 1987;

Getchell, 1986; Getchell et al., 1984; Lancet, 1986; Lancet and Pace, 1987). These cilia can be detached from frog olfactory epithelium via a calcium shock and harvested after centrifugation on a sucrose cushion (Anholt et al., 1986; Chen and Lancet, 1984; Chen et al., 1986a). The resulting preparation is enriched in adenylate cyclase activity and G_s . It contains virtually no detectable G_i , a G-protein prominent in homogenates of olfactory epithelium, probably localized to glands (Anholt et al., 1987). [G-Proteins are guanine nucleotide-binding regulatory proteins; G_s and G_i mediate the stimulation and inhibition, respectively, of adenylate cyclase; G_o is a related G-protein prominent in brain and of unknown function.]

A 260 kDa glycoprotein that binds wheat germ agglutinin (WGA) and is found in olfactory axons may represent the α subunit of the voltage-dependent sodium channel and also is not detected in isolated olfactory cilia (Farmer and Anholt, unpublished). Both the α and β subunits of tubulin are prominent in the cilia. These characteristics indicate a substantial degree of purity of this chemosensory cilia preparation.

Electron micrographs show that the cilia are fragmented during isolation and that their plasma membranes tend to separate from the axonemal core, forming 100-500 nm ciliary membrane vesicles (Anholt et al., 1986). The ciliary membranes isolated from the olfactory epithelium are expected to contain all the membrane-associated components that participate in olfactory transduction, including odorant-activated ion channels and an odorant-sensitive adenylate cyclase.

III. ACTIVATION BY ODORANTS OF ION CHANNELS IN OLFACTORY CILIA

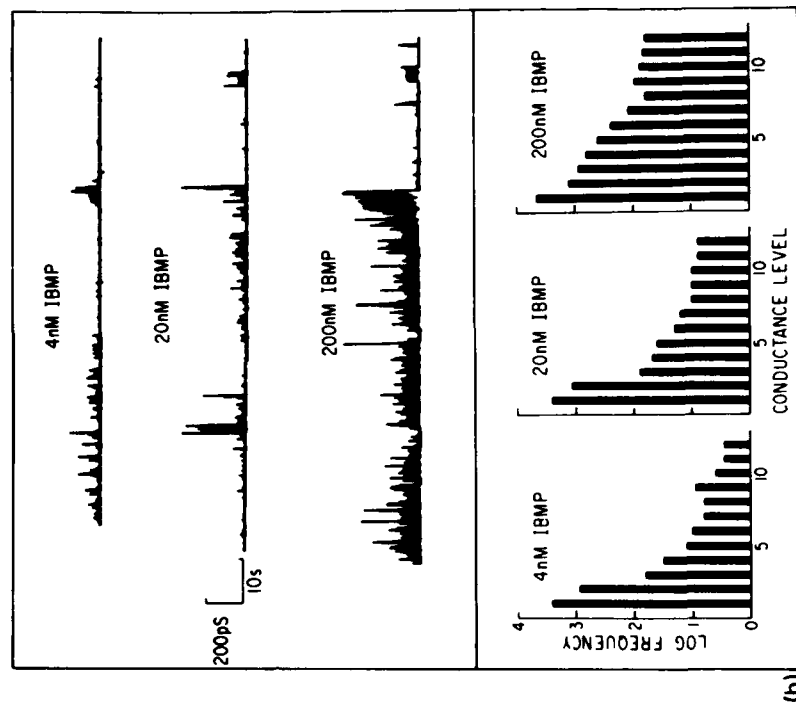
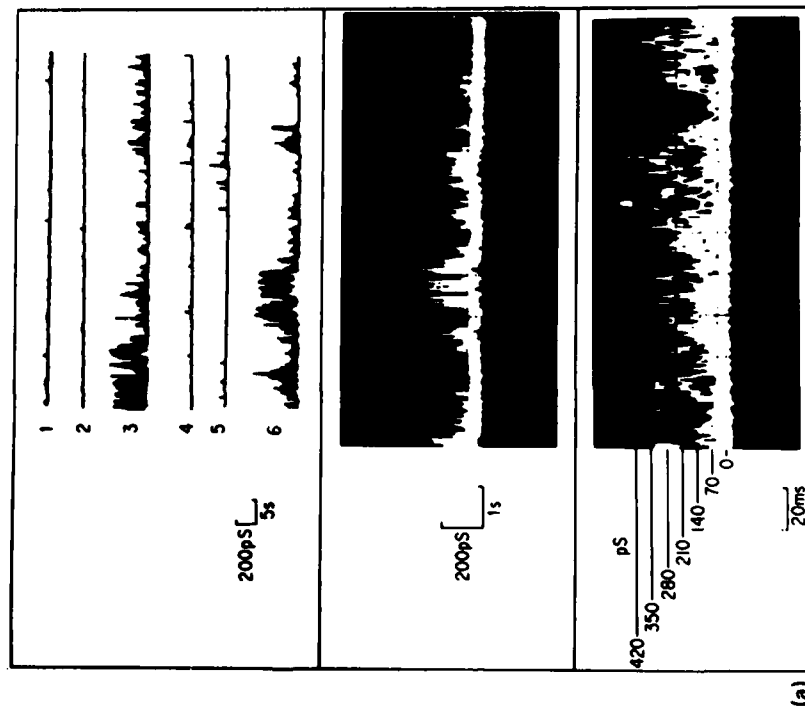
Odorant-activated channels can be detected after fusion of membranes from olfactory cilia to planar lipid bilayers (Labarca et al., 1988). The ciliary membranes are equilibrated in 0.5 M sucrose and fused to the bilayer by the addition of calcium and, if necessary, an osmotic gradient of KCl. The planar bilayers are formed across a 200-400 μ m hole in a polycarbonate septum from a mixture of 20 mg of phosphatidylethanolamine (from *Escherichia coli*) and 20 mg of phosphatidylserine (from bovine brain) per milliliter of *n*-decane. After fusion, excess calcium and KCl can be removed by perfusion of the chamber and single channels can be recorded via electrodes placed in each compartment.

A variety of single channels are observed after fusion of olfactory cilia to the planar bilayer in 0.2 M salt-containing buffer. These include a 190 pS Ca^{2+} -activated K channel, which resembles the 130 pS Ca^{2+} -activated K channel observed in patch-clamp studies on the dendritic knob and soma of isolated murine olfactory receptor cells (Maue and Dionne, 1987). A 40 pS



<input checked="" type="checkbox"/>
<input type="checkbox"/>
<input type="checkbox"/>
Codes

1st	Special
A-1	



cation-selective channel of unknown function is also found. Replacement of chloride ions by acetate ions and substitution of potassium by sodium abolishes the appearance of large conductance Cl^- channels and the 190 pS K channel, and allows the detection of odorant-activated channels. Addition of nanomolar concentrations of the bell pepper odorant, 3-isobutyl,2-methoxypyrazine (IBMP), or the citrus odorant, citralva (3,7-dimethyl-2,6-octadienenitrile), causes bursts of single-channel fluctuations. Analysis of these bursts reveals that they consist of discrete conductance levels that appear as multiples of a 35 pS conductance (Fig. 2a). Increasing the odorant concentration increases the duration and frequency of the bursts as well as the probability of populating higher conductance levels (Fig. 2b). Odorant-induced channel activation is reversible by removal of the odorant via perfusion of the bilayer chamber (Fig. 2a, top). The odorant-activated channels are cation-

FIGURE 2 Activation by odorants of ion channels. Single channels were recorded after fusion of ciliary membranes from the olfactory epithelium of the bullfrog to planar lipid bilayers. The records were obtained from bilayers bathed in symmetrical solutions of 0.2 M sodium acetate, 5 mM HEPES, 0.5 mM EGTA, pH 7.0, at an applied voltage of 50 mV. Upward deflections represent channel openings. (a): (a) Reversible activation of channels by the bell pepper odorant, 3-isobutyl,2-methoxypyrazine (IBMP). Traces 1 and 2 show the conductance after fusion of ciliary membranes to the bilayer before addition of the odorant. The third trace was obtained 1 minute after the addition of 40 nM IBMP to the trans compartment. The fourth and fifth traces were obtained after perfusion of the chamber with odorant-free buffer. Note the reversal of the activation. The last trace was obtained 1 minute after a second addition of 40 nM odorant. (b) Oscilloscope trace of the odorant-sensitive channel. Note the bursting behavior. Discrete bursts last several hundred milliseconds and display multiple conductance levels. The record was obtained 1 minute after the addition of 30 nM IBMP and filtered at 500 Hz. (c) Superposition of several fast sweeps of the odorant-sensitive channel. Note the defined conductance levels at intervals of 35 pS. (b): Effect of odorant concentration on channel activation. (a) Channel activation by increasing concentrations of IBMP. The records were obtained from the same membrane 2 minutes after subsequent additions of 4, 20, and 200 nM of the odorant and filtered at 500 Hz. (b) Frequency conductance histograms of the odorant-activated channel at 4, 20, and 200 nM of IBMP. The frequencies of channel opening to the different conductance levels were obtained by computer analysis of records similar to those shown in (a). Each histogram contains 3 minutes of data analysis beginning 2 minutes after the addition of the odorant. The indicated conductance levels are integral multiples of a 35 pS unitary conductance. Note that increasing the concentration of odorant leads to an increased number of bursts, a longer duration of individual bursts, and an increase in the frequency with which the higher conductance levels are observed. (Modified from Labarca et al., 1988.)

selective and have millisecond open times. They are not observed in bare lipid bilayers or bilayers to which cilia from respiratory epithelium have been fused. Their kinetic behavior is highly complex, and it is not clear whether the observed bursting behavior reflects cooperative opening of discrete units or sequential conformational transitions within a single-channel moiety triggered by binding of the odorant. It also remains to be determined whether different odorant-binding sites are linked to the same or to different channels and whether different odorants induce different channel kinetics.

It is of interest to note that olfactory receptor cells have a high membrane impedance of 3-6 G Ω (Firestein and Werblin, 1987; Trotier, 1986). Hence only picoamperes of injected current are sufficient to depolarize the cells to threshold (Firestein and Werblin, 1987; Hedlund et al., 1987). This implies that a single burst of channel activity to the higher conductance levels may result in a depolarizing current that is large enough to cause excitation of the olfactory receptor cell. Thus, effective cellular control mechanisms must exist to monitor the gating of ion channels by odorants and to regulate the fraction of activatable channels. It is tempting to speculate that cyclic nucleotides and calcium may be involved in such control mechanisms.

IV. ADENYLATE CYCLASE OF OLFACTORY RECEPTOR CELLS

Isolated olfactory cilia of frog and rat contain a high activity of adenylate cyclase and its regulatory G-protein, G_s (Anholt et al., 1987; Pace et al., 1985; Shirley et al., 1986; Sklar et al., 1986). In addition, they contain G_o (Anholt et al., 1987). The adenylate cyclase is stimulated by some, predominantly hydrophobic, odorants in a GTP-dependent manner (Pace et al., 1985; Shirley et al., 1986; Sklar et al., 1986). In contrast to the activation of odorant-sensitive channels, stimulation of adenylate cyclase requires micromolar to millimolar concentrations of odorant, sometimes near the aqueous solubility limit of the compound being tested. It has been suggested that, by analogy to hormonal systems, odorants activate the adenylate cyclase via receptor proteins (Lancet, 1986; Lancet and Pace, 1987). A 95 kDa WGA-binding protein, enriched in the cilia, has been proposed to represent a collection of putative odorant receptors (Chen et al., 1986b; Lancet, 1986; Lancet and Pace, 1987). However, binding of odorants to this protein has not yet been reported. Alternatively, it is possible that partitioning of lipophilic odorants at these high concentrations in the membrane causes some dissociation of the subunits of G_s, and the resulting liberation of a small amount of G_s may account for the observed increase in cAMP generation (Anholt, 1987 and 1988; Lerner et al., 1988).

One approach toward the designation of specific functions to defined proteins of the olfactory membrane is through the use of functional recon-

stitution studies. The olfactory adenylate cyclase can be solubilized by treating olfactory cilia with Lubrol PX in the presence of supplementary soybean lipid as well as forskolin and sodium fluoride (Anholt, 1988). When solubilized in an activated form and protected by exogenous lipid, the enzyme retains its activity and can be incorporated into liposomes via removal of the detergent. The reconstituted adenylate cyclase is intact, since forskolin activates it with the same EC_{50} as the native enzyme (1-2 μ M; Fig. 3a). However, the EC_{50} for activation by $GTP\gamma S$, a nonhydrolyzable analogue of GTP, has shifted to a 350-fold higher concentration in reconstituted membranes (Fig. 3b) and the reconstituted enzyme is no longer stimulated by odorants. This may reflect either denaturation of a putative odorant receptor and/or partial impairment of the G-protein during the solubilization and reassembly processes, or an altered interaction between the adenylate cyclase and G_s as a result of the lowered packing density of both components in the reconstituted membrane. Measurements of enhancement by odorants of the rate of GTP hydrolysis in native and reconstituted membranes may help resolve this question. Eventually, the molecular dissection of the olfactory membrane through functional reconstitution studies may identify proteins that perform specific functions during olfactory transduction.

In addition to adenylate cyclase on the cilia, adenylate cyclase activity is found on axonal membranes from homogenates of the olfactory nerve, which in the bullfrog can easily be dissected. The specific activity of the axonal adenylate cyclase is approximately 25-fold lower than the specific activity found in the dendritic cilia. The axonal enzyme differs from the ciliary enzyme in that it is not sensitive to odorants and requires 20-fold higher concentrations of $GTP\gamma S$ for activation via its G-protein (Fig. 4). In these aspects, the axonal adenylate cyclase resembles the reconstituted ciliary enzyme. Western blots, using monospecific antisera, reveal both G_s and G_o on axonal membranes, the latter being particularly prominent (Farmer and Anholt, unpublished). Axonal membranes also contain a group of WGA-binding proteins with apparent molecular weights around 95 kDa, previously believed to be located only on the cilia and proposed to represent odorant receptors (Chen and Lancet, 1984; Chen et al., 1986b). However, higher concentrations of WGA are necessary to detect these proteins in axonal membranes than in ciliary membranes (Farmer and Anholt, unpublished).

Axons of olfactory receptor cells do not synapse on their way from the olfactory epithelium to the central nervous system. It is, therefore, surprising to find adenylate cyclase activity on the axons. In fact, adenylate cyclase activity is hardly detectable in membranes derived from axons of the sciatic nerve and G_s is not detected in these membranes on Western blots. The presence of adenylate cyclase on olfactory axons raises the question of which factors stimulate the axonal enzyme. It is possible that axons within individual

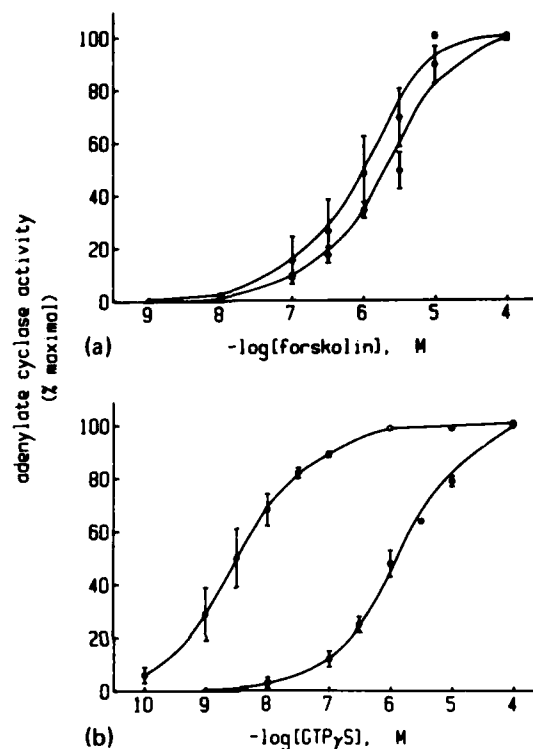


FIGURE 3 Stimulation of the olfactory adenylate cyclase by forskolin and GTP γ S in native and reconstituted membranes. (a) Stimulation by forskolin in native (left hand curve) and reconstituted (right hand curve) membranes. The EC_{50} values for stimulation by forskolin are 1.0 ± 0.4 and 2.1 ± 0.4 μ M for native and reconstituted membranes, respectively. (b) Stimulation by GTP γ S in native (left hand curve) and reconstituted (right hand curve) membranes. The EC_{50} values for stimulation by GTP γ S are 4.0 ± 0.5 nM and 1.4 ± 0.3 μ M for native and reconstituted membranes, respectively. Data points represent the average \pm SEM of three experiments, each consisting of triplicate measurements. In native membranes, addition of odorants, such as citralva (3,7-dimethyl-2,6-octadienenitrile), 3-isobutyl,2-methoxypyrazine, and methone, at concentrations of 0.1 mM in the presence of GTP, leads to a 50-85% increase of adenylate cyclase activity above the basal rate. Reconstituted membranes do not show sensitivity to odorants. Compare the behavior of the reconstituted adenylate cyclase of olfactory cilia with the properties of the axonal adenylate cyclase, illustrated in Fig. 4. (From Anholt, 1988.)

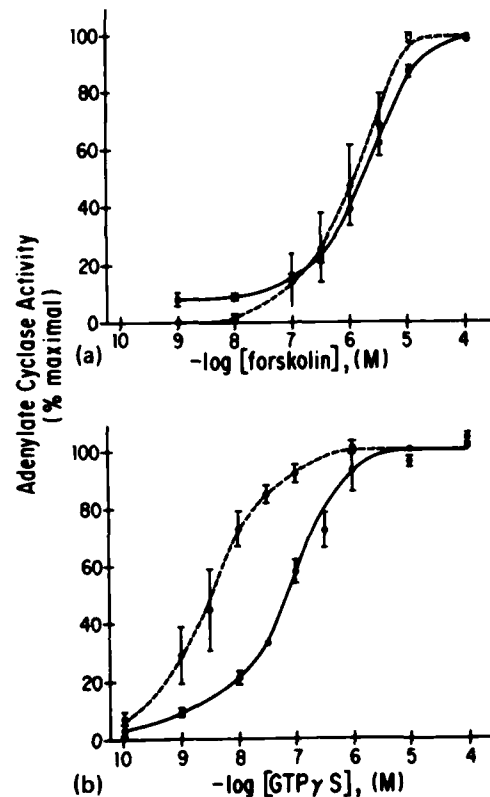


FIGURE 4 Stimulation of ciliary (○, ----) and axonal (●, —) adenylate cyclase of olfactory receptor cells by forskolin and GTP γ S. (a) Stimulation by forskolin. The EC₅₀ values for cilia and axons are 1.1 ± 0.3 and 2.0 ± 0.1 μ M, respectively. (b) Activation by GTP γ S. The EC₅₀ values for cilia and axons are 4.5 ± 0.7 and 79 ± 10 nM, respectively. Data points represent the average \pm SEM of three experiments, each consisting of triplicate measurements. Like the reconstituted ciliary enzyme, the axonal adenylate cyclase is not stimulated by odorants.

nerve fascicles release agents that influence the activity of adjacent axons. Alternatively, internal cellular modulators (e.g., calmodulin) could regulate the intracellular cAMP level by stimulating or inhibiting the adenylate cyclase. The presence of adenylate cyclase on both processes of the olfactory neuron, the identification of cAMP-activated currents on the cilia, the dendritic knob, and the soma (Nakamura and Gold, 1987), and the modulation

by forskolin of ionic currents recorded from the soma and the dendritic knob of olfactory receptor cells (Maue and Dionne, 1987; Trotier and MacLeod, 1987) suggest that cAMP acts as a neuromodulator throughout the olfactory receptor cell, regulating both transduction at the chemosensory cilia and the resulting transfer of encoded odor information by olfactory axons from the periphery to the brain.

The different potencies with which GTP _{γ} S stimulates the ciliary adenylate cyclase and the axonal adenylate cyclase raises the question of whether the cilia contain a distinct subtype of G_s with an intrinsically higher affinity for GTP. Alternatively, it is possible that G_s in the cilia is identical to G_s in the axons, but that it interacts more effectively with the ciliary adenylate cyclase due to the higher concentration of this enzyme in the membrane. A detailed examination of the pharmacological properties of G_s in the cilia and in the axons, such as the potency with which GTP analogues can inhibit the rate of GTP hydrolysis, will help resolve this question.

V. CALCIUM AS A REGULATOR OF THE RESPONSE TO ODORANTS

Results from electrophysiological experiments indicate that calcium may play an important role in the response to odorants (Maue and Dionne, 1987; Suzuki, 1978; Trotier, 1986). The concentration of calcium in the mucus is in the millimolar range, and its intracellular concentration has been estimated to be below 0.1 μ M (Maue and Dionne, 1987). Although the calcium concentration inside the cilia has not been measured, it is evident that a chemical gradient of several orders of magnitude exists for calcium across the chemosensory membrane, much larger than for any of the monovalent ions. Removal of any permeability barrier to calcium would result in a rapid rise of the intraciliary calcium concentration.

A rise in the intraciliary free calcium concentration would affect a variety of cellular parameters. Calcium inhibits the olfactory adenylate cyclase (Fig. 5; Shirley et al., 1986; Sklar et al., 1986) and activates K channels (Firestein and Werblin, 1987; Labarca et al., 1988; Maue and Dionne, 1987). Activation of K channels is maximal at 1.0 μ M calcium (Maue and Dionne, 1987), whereas inhibition of adenylate cyclase is half-maximal at 100-200 μ M calcium (Shirley et al., 1986; Sklar et al., 1986). Olfactory receptor cells contain a calcium-dependent ATPase that may transport calcium out of the cell (Farmer, Karavanich, and Anholt, unpublished). The activity of this ATPase in the dendritic cilia is twice as high as its activity in axonal membranes of olfactory receptor cells. Immunochemical characterization of this enzyme indicates that it is distinct from the ATPase of muscle sarcoplasmic reticulum, but resembles the cardiac slow-twitch ATPase. Activation of this

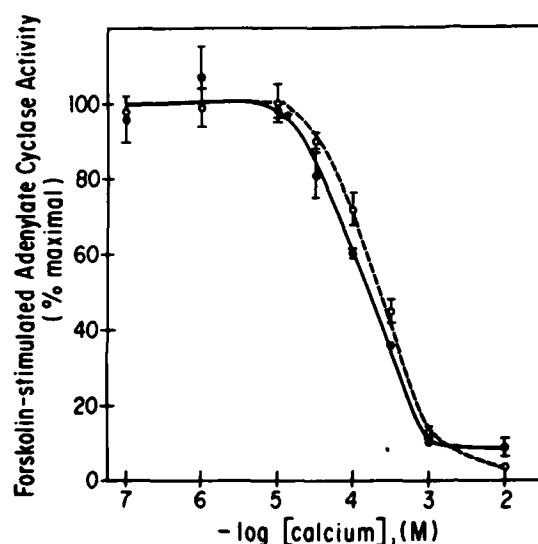


FIGURE 5 Inhibition of adenylate cyclase of olfactory cilia (O, ----) and olfactory axons (●, —) by calcium. The IC_{50} values for cilia and axons are 200 ± 9 and $141 \pm 7 \mu M$, respectively. All assays were performed in the presence of $10 \mu M$ forskolin. Data points represent the average \pm SEM of three experiments, each consisting of triplicate measurements.

enzyme by calcium is half-maximal at 10-20 nM and maximal at 100 nM free calcium.

Calcium also regulates the phosphorylation of proteins in olfactory receptor cells. When olfactory cilia are incubated with low concentrations of [^{32}P] γ -ATP (10 nM) in the presence of EGTA or EDTA, phosphorylation of a 48 kDa protein is observed. Phosphorylation of this protein occurs within 1 minute and is blocked by micromolar concentrations of calcium. We have named this protein CaRPP48 (calcium-regulated phosphoprotein of 48 kDa). CaRPP48 is less prominent in axonal membranes than in ciliary membranes, and its phosphorylation appears not to be affected by cyclic nucleotides. The function of CaRPP48 is not known, but its cyclic AMP-independent phosphorylation, peripheral association with the membrane and apparent molecular weight raise the possibility that CaRPP48 may represent a neuronal growth associated problem, identical or similar to GAP43/B50 (Verhaagen et al., 1989). Further studies will determine whether inhibition by calcium of the phosphorylation of CaRPP48 is due to inhibition of a kinase, detachment of a kinase from the membrane, or stimulation of a phosphatase.

Studies on the role of calcium in olfaction have thus far been limited. However, it is clear that variations in the cytoplasmic free calcium concentration in the cilia will elicit a pleiotropic spectrum of effects relevant to the regulation of the response of the olfactory receptor cell to odorants. The exact ways of entry of calcium into the cell as a result of olfactory transduction events remain to be established. It is possible that calcium could enter via the odorant-activated cation channel or via cyclic nucleotide-activated channels. The permeability of these channels to calcium remains, however, to be investigated.

VI. REGULATION OF OLFACTORY TRANSDUCTION

It has been suggested that binding of odorants to receptors results in the formation of cyclic AMP, which would then open ion channels to initiate excitation of the olfactory receptor cell (Gold and Nakamura, 1987; Gold et al., Chapter 15; Lancet, 1986; Chapter 13; Lancet and Pace, 1987). However, the nature of odorant receptors is still enigmatic, and direct evidence linking odorant binding to excitation of the olfactory receptor cell via a cAMP-mediated pathway is still lacking.

An alternative role for cAMP in olfaction that is consistent with currently available experimental evidence would be regulation of the intraciliary calcium concentration. Signal transduction at low concentrations of odorants would be mediated via odorant-activated channels and at higher concentrations, or upon prolonged exposure to odorants, cAMP would be generated. cAMP would then facilitate the entry of extracellular calcium, perhaps via cyclic nucleotide-activated channels. The subsequent rise in the intraciliary calcium concentration would inhibit further generation of cAMP via the olfactory adenylate cyclase and activate K channels to repolarize the membrane. The sodium/potassium-dependent ATPase (Anholt et al., 1986) and the calcium-dependent ATPase would subsequently restore the ion distribution across the ciliary membrane. Thus, activation by odorants of ion channels would underlie the phasic component of the EOG, whereas activation of adenylate cyclase may give rise to the tonic component of the EOG. It is also possible that cAMP regulates the membrane potential of the olfactory receptor cell through phosphorylation of ion channel proteins.

Elevated intraciliary calcium concentrations may account for the refractory period (desensitization) observed after repeated application of odor pulses to the epithelium. These events would be accompanied by calcium-regulated (Farmer and Anholt, unpublished) and cAMP-regulated phosphorylation (Heldman and Lancet, 1986) and dephosphorylation reactions of proteins associated with the transduction process. It should, however, be emphasized that further experimentation is required to validate any of these

speculations and that models and working hypotheses regarding olfactory transduction can be expected to undergo frequent revision and modification as new data become available in this rapidly evolving field.

ACKNOWLEDGMENTS

This work has been supported by U.S. Public Health Service grant NS-24521, U.S. Army Research Office grant DAAL03-86-K-0130, and Office of Naval Research grant S4411107. We thank Dr. Sidney A. Simon for helpful comments and valuable suggestions during the preparation of the manuscript.

REFERENCES

- Adamek, G. D., Gesteland, R. C., Mair, R. G., and Oakley, B. (1984). Transduction physiology of olfactory receptor cells. *Brain Res.* 310: 87-97.
- Anholt, R. R. H. (1987). Primary events in olfactory reception. *Trends Biochem. Sci.* 12: 58-62.
- Anholt, R. R. H. (1988). Functional reconstitution of the olfactory membrane: Incorporation of the olfactory adenylate cyclase in liposomes. *Biochemistry* 27: 6464-6468.
- Anholt, R. R. H., Aebi, U., and Snyder, S. H. (1986). A partially purified preparation of isolated chemosensory cilia from the olfactory epithelium of the bullfrog, *Rana catesbeiana*. *J. Neurosci.* 6: 1962-1969.
- Anholt, R. R. H., Mumby, S. M., Stoffers, D. A., Girard, P. R., Kuo, J. F., and Snyder, S. H. (1987). Transduction proteins of olfactory receptor cells: Identification of guanine nucleotide binding proteins and protein kinase C. *Biochemistry* 26: 788-795.
- Bronshtein, A. A., and Minor, A. V. (1977). The regeneration of olfactory flagella and restoration of electroolfactogram after the treatment of the olfactory mucosa with Triton X-100. *Tsitologiya* 19: 33-39.
- Chen, Z., and Lancet, D. (1984). Membrane proteins unique to vertebrate olfactory cilia: Candidates for sensory receptor molecules. *Proc. Natl. Acad. Sci. USA* 81: 1859-1863.
- Chen, Z., Pace, U., Heldman, J., Shapiro, A., and Lancet, D. (1986a). Isolated frog olfactory cilia: A preparation of dendritic membrane from chemosensory neurons. *J. Neurosci.* 6: 2146-2154.
- Chen, Z., Pace, U., Ronen, D., and Lancet, D. (1986b). Polypeptide gp95: A unique glycoprotein of olfactory cilia with transmembrane receptor properties. *J. Biol. Chem.* 261: 1299-1305.
- Firestein, S., and Werblin, F. S. (1987). Gated currents in isolated olfactory receptor neurons of the larval tiger salamander. *Proc. Natl. Acad. Sci. USA* 84: 6292-6296.
- Getchell, T. V. (1986). Functional properties of vertebrate olfactory receptor neurons. *Physiol. Rev.* 66: 772-818.

- Getchell, T. V., Margolis, F. L., and Getchell, M. L. (1984). Perireceptor and receptor events in vertebrate olfaction. *Prog. Neurobiol.* 23: 317-345.
- Gold, G. H., and Nakamura, T. (1987). Cyclic nucleotide-gated conductances: A new class of ion channels mediates visual and olfactory transduction. *Trends Pharmacol. Sci.* 8: 312-316.
- Hedlund, B., Masukawa, L. M., and Shepherd, G. M. (1987). Excitable properties of olfactory receptor neurons. *J. Neurosci.* 7: 2338-2343.
- Heldman, J., and Lancet, D. (1986). Cyclic AMP-dependent protein phosphorylation in chemosensory neurons: Identification of cyclic nucleotide-regulated phosphoproteins in olfactory cilia. *J. Neurochem.* 47: 1527-1533.
- Kurihara, K., and Koyama, N. (1972). High activity of adenyl cyclase in olfactory and gustatory organs. *Biochem. Biophys. Res. Commun.* 48: 30-33.
- Labarca, P., Simon, S. A., and Anholt, R. R. H. (1988). Activation by odorants of a multistate cation channel from olfactory cilia. *Proc. Natl. Acad. Sci. USA* 85: 944-947.
- Lancet, D. (1986). Vertebrate olfactory reception. *Annu. Rev. Neurosci.* 9: 329-355.
- Lancet, D., and Pace, U. (1987). The molecular basis of odor recognition. *Trends Biochem. Sci.* 12: 63-66.
- Lerner, M. R., Reagan, Y., Gyorgyi, T., and Roby, A. (1988). Olfaction by melanophores: What does it mean? *Proc. Natl. Acad. Sci. USA* 85: 261-264.
- Maue, R. A., and Dionne, V. E. (1987). Patch-clamp studies of isolated mouse olfactory receptor neurons. *J. Gen. Physiol.* 90: 95-125.
- Menevse, A., Dodd, G., and Poynder, T. M. (1977). Evidence for the specific involvement of cyclic AMP in the olfactory transduction mechanism. *Biochem. Biophys. Res. Commun.* 77: 671-677.
- Minor, A. V., and Sakina, N. L. (1973). The role of cyclic adenosine-3',5'-monophosphate in olfactory reception. *Neurofiziologiya* 5: 415-422.
- Nakamura, T., and Gold, G. H. (1987). A cyclic nucleotide-gated conductance in olfactory receptor cilia. *Nature* 325: 442-444.
- Ottoson, D. (1956). Analysis of the electrical activity of the olfactory epithelium. *Acta Physiol. Scand.* 35 (suppl 122): 1-83.
- Pace, U., Hanski, E., Salomon, Y., and Lancet, D. (1985). Odorant-sensitive adenylate cyclase may mediate olfactory reception. *Nature* 316: 255-258.
- Shirley, S. G., Robinson, C. J., Dickinson, K., Aujla, R., and Dodd, G. H. (1986). Olfactory adenylate cyclase of the rat: Stimulation by odorants and inhibition by Ca^{2+} . *Biochem. J.* 240: 605-607.
- Sklar, P. B., Anholt, R. R. H., and Snyder, S. H. (1986). The odorant-sensitive adenylate cyclase of olfactory receptor cells: Differential stimulation by distinct classes of odorants. *J. Biol. Chem.* 261: 15538-15543.
- Suzuki, N. (1978). Effects of different ionic environments on the responses of single olfactory receptors in the lamprey. *Comp. Biochem. Physiol.* 61A: 461-467.
- Tagaki, S. F., Wyse, G. A., Kitamura, H., and Ito, K. (1968). The roles of sodium and potassium ions in the generation of the electroolfactogram. *J. Gen. Physiol.* 51: 522-578.

MOLECULAR INTERACTIONS AT THE CILIARY MEMBRANE 361

- Tagaki, S. F., Kitamura, H., Imai, K., and Takeuchi, H. (1969). Further studies on the roles of sodium and potassium in the generation of the electroolfactogram. *J. Gen. Physiol.* 53: 115-130.
- Trotier, D. (1986). A patch-clamp analysis of membrane currents in salamander olfactory receptor cells. *Pflügers Arch.* 407: 589-595.
- Trotier, D., and MacLeod, P. (1987). The amplification process in olfactory receptor cells. *Ann. NY Acad. Sci.* 510: 677-679.
- Verhaagen, Y., Oestreicher, A. B., Gispen, W. H., and Margolis, F. L. (1989). The expression of the growth associated protein B50/GAP43 in the olfactory system of neonatal and adult rats. *J. Neurosci.* 9: 683-691.